

Title: **NITROGEN, AMMONIA TEST**

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1.0 OBJECTIVE

This method measures the amount of ammonia present in sample from acute and chronic bioassay tests.

2.0 HEALTH AND SAFETY

Personnel should wear lab coats, safety goggles, and chemical resistant gloves.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

This method should be restricted to use by or under the supervision of professionals experienced in aquatic toxicity testing.

4.0 REQUIRED AND RECOMMENDED MATERIALS

HACH DR/700 Colorimeter
Alkaline Cyanurate Reagent
Volumetric pipets
Eppendorf Pipetor and tips
Deionized (DI) water
Tap water
pH test strips

Salicylate Reagent
10-mL or 25-mL Sample Cells
Pipet bulbs
Sharpie
Gallon jar
10% HCl

5.0 PROCEDURE

5.1 Sample Collection

- Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.
- Using a volumetric pipet, add the appropriate sample to the cells.
- **NOTE:** For the juvenile clam assay, the 10-mL cells are used. Pull 1 mL of sample and dilute up to the 10-mL mark with DI water.

5.2 Testing

- Install module number 61.01 in a DR/700.
- Press **I/O**. The display will show **610 nm** and module number **61.01**.
- After two seconds, the display will show a program number, the concentration units and the zero prompt. If necessary, press the **UP** arrow until the lower display shows program number **61.061**.
- Label the sample cells with the appropriate sample identifications and a blank.
- Fill a 25-mL cell to the 25-mL mark with the sample. Continue this with all samples.
- Fill a 25-mL cell to the 25-mL mark with the deionized water for the blank.
- Add the contents of one Salicylate Reagent Powder Pillow to each cell. Cap and invert the cells several times to mix.
- Wait 3 minutes.
- Add the contents of one Alkaline Cyanurate Reagent Powder Pillow to each cell. Cap and invert the cells several times to mix. **NOTE:** A green color will develop if ammonia nitrogen is present.

- Wait 15 minutes.
- Place the blank into the cell holder. **NOTE:** Typical indoor lighting permits the DR/700 to operate with the cell compartment cover open. In bright sunlight it may be necessary to close the cell compartment cover. Transfer 10-ml of the blank solution to a 10-mL cell. If the 10-mL cell is used for the blank, another 10-mL cell must be used for the sample.
- Press **ZERO**. The display will count down from 20 to 0. Then the display will show 0.00 mg/L and the zero prompt will turn off.
- Place the prepared sample in the cell holder. **NOTE:** Typical indoor lighting permits the DR/700 to operate with the cell compartment cover open. In bright sunlight it may be necessary to close the cell compartment cover. Transfer 10-ml of the blank solution to a 10-mL cell. If the 10-mL cell is used for the blank, another 10-mL cell must be used for the sample.
- Press **READ**. The display will count down from 20 to 0. Then the display will show the results in mg/L NH_3 as N.
- **NOTE:** A flashing display of the concentration range maximum value is an indication that the reading was beyond the upper end of the factory-entered calibrated range. A sample dilution (prior to treatment) may be necessary to bring the concentration within the range of the colorimeter. If a diluted sample is measured, multiply the test result by the dilution factor.
- **NOTE:** A flashing minimum concentration value indicates that the sample measured had a concentration value less than zero. It may be caused by a bad choice of blank solution or by samples cells poorly matched.
- To convert results to other units see Table 1.

5.3 Neutralization of Spent Ammonia Test Reagents

- Place spent ammonia reagent container in sink. Pour into gallon jar until half full.
- Check pH with a test strip only. Do not use pH meter of any type. The pH of the spent reagent will be highly alkaline.
- Using 10% hydrochloric acid (HCl), from glassware acid bath if necessary, carefully pour into gallon jar with spent reagent. About 500 ml at a time will be sufficient. Stir until well mixed.

- Check pH again. Add 10% HCl until pH of spent reagent is 7.0.
- Once pH is 7.0, flush down sink with copious amounts of tap water.
- Repeat steps until all spent ammonia reagent has been neutralized.
- Rinse spent reagent containers and clean according to labware SOP.

6.0 QUALITY ASSURANCE/QUALITY CONTROL

Personnel should adhere to good laboratory practices.

7.0 REFERENCES

HACH DR/700 Colorimeter Manual.

8.0 TABLES

Table 1. Conversion Factors

To convert reading from	To	Multiply by
mg/L $\text{NH}_3\text{-N}$	mg/L NH_4^+	1.29
mg/L $\text{NH}_3\text{-N}$	mg/L NH_3	1.22